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Characterization of prostanoid relaxant/inhibitory receptors (ψ) using a highly selective agonist, TR4979

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1 TR4979, an analogue of prostaglandin E_1 (PGE_1) was evaluated on respiratory and non-respiratory isolated tissues known to contain heterogeneous or homogeneous populations of the two classes of prostanoid (prostaglandins and thromboxanes) receptors. These receptors are classified as 'χ' the contractant/stimulant receptor with '$\chi_{1,2,3}$' being three subdivisions and 'ψ' the relaxant/inhibitory receptor(s).

2 On a respiratory tissue (cat trachea) containing predominantly 'ψ'-receptors, TR4979 was 26 times less potent than PGE_1 or PGE_2.

3 On other respiratory tissues known to contain mixtures of the '$\chi_{1,2,3}$'- and 'ψ'-receptors (guinea-pig trachea and lung strip, cat lung strip and human bronchial muscle), TR4979 consistently acted as a potent relaxant whereas PGE_2 and to a lesser extent PGE_1 had significant contractant activities.

4 Human pregnant uterus, guinea-pig and rat pseudo-pregnant uteri, rat colon and fundic strips and chick ileum are known to contain one or more of the three subclasses of the 'χ'-receptor. TR4979 (10^{-9}–10^{-5}M) was inactive on all these tissues whereas all of the reference prostanoids were contractants of varying potencies.

5 PGE_1 and histamine-induced contractions of the guinea-pig isolated ileum were both non-competitively antagonized by increasing concentrations of TR4979 suggesting that 'ψ'-receptors also exist on this tissue.

6 TR4979 is a highly selective agonist of prostanoid 'ψ' (relaxant/inhibitory)-receptors which at present have been demonstrated to exist mainly in the lung. This prostaglandin analogue is a useful new selective pharmacological tool for revealing as yet unidentified prostanoid 'ψ'-receptors and actions in a wide range of non-respiratory tissues/organs such as the guinea-pig ileum.

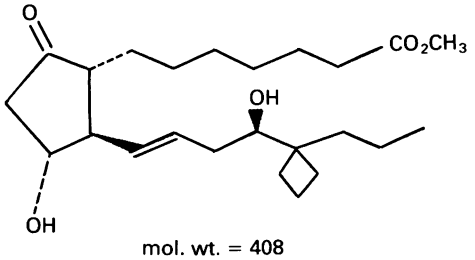
Introduction

Prostaglandin E_2 (PGE_2) and PGE_1 were originally shown by Cuthbert (1969) to act as bronchodilator agents when inhaled by asthmatics. Such studies stimulated intense interest in the potential of prostaglandins as new therapeutic agents for the treatment of airways disease. A large number of analogues with bronchodilator activity were subsequently evaluated in man but only one has as yet consistently acted as a bronchodilator (Grudzinskas *et al.*, 1980; Nizankowska *et al.*, 1985). Further studies on isolated tissues revealed that the ubiquitous prostaglandins, and to a much lesser extent their fellow arachidonate metabolites the thromboxanes, have multiple biological actions. Some of these actions counteract each other, as demonstrated with PGE_2 on human isolated bronchial muscle, consequently the need for selectivity of action was critical for the development of any prostanoid (prostaglandins and Thromboxanes) agonist/antagonists (Svensson *et al.*, 1976; 1977; Gardiner & Collier, 1980).

In an attempt to identify such selective agonists/antagonists of the prostanoid actions in the lungs and other organs, a number of groups investigated whether specific receptors exist for these agents (Coleman *et al.*, 1980a; Gardiner & Collier, 1980; Jones *et al.*, 1982). In the absence of any selective antagonists, early studies were performed using a range of prostaglandins and rank orders of agonist potency were determined on many tissues. Despite the interpretation of such studies being fraught with numerous difficulties including differences in the agonists diffusion, uptake and/or metabolism etc., the first attempt at a putative characterization and classification of receptors in the airways was proposed by Gardiner & Collier (1980) and modified (Gardiner & Browne, 1984). Receptor classes were identified according to the action of the

prostaglandin, i.e. Class 1 'χ' receptors for contractant/stimulant effects, Class II 'ψ' receptors for relaxant/inhibitory effects. This classification differs from that recently proposed by Coleman *et al.* (1984) in which each prostanoid was classified as having its own receptor class. Thromboxane A_2 (TXA_2) was not assessed in the earliest studies (Gardiner & Collier, 1980) but was shown by Jones *et al.* (1982) and Coleman *et al.* (1981) (using the thromboxane mimetic U46619, PGH_2 and TXA_2) to be a potent contractant of a wide range of tissues. Prostaglandins were also potent contractants on many of these tissues, suggesting that TXA_2 might usefully be classified as acting through 'χ'-receptors. The identification of selective antagonists of the thromboxane mimetic U46619 (i.e. EP045) and of some prostaglandin contractant effects (i.e. SC19220) has led to the incorporation of the suggestions of Coleman *et al.* (1984) into the original classification (Gardiner & Collier, 1980). The new classification retains 'χ'-receptors but they are subdivided on the basis of selective agonists and antagonists accordingly, 'χ_1' (agonist TXA_2, antagonist EP045) 'χ_2' (agonist $PGF_{2\alpha}/PGD_2$) and 'χ_3' (agonist PGE_2, antagonist SC19220) (Scanner, 1969; Bennett & Posner, 1971; Jones *et al.*, 1982).

TR4979 is an analogue of PGE_1 (see Figure 1) which was initially identified as a bronchodilator agent. The following series of studies evaluated the selectivity of this analogue for prostaglandin 'ψ'-receptors, using a bank of respiratory and non-respiratory tissues known to contain homogeneous or heterogeneous populations of the two classes of prostanoid receptors.



mol. wt. = 408

Figure 1 The chemical structure of TR4979.

Methods

(1) *Respiratory isolated tissues containing prostaglandin 'ψ' (relaxant)-receptors*

Guinea-pig trachea Male Dunkin Hartley guinea-pigs, 350–450 g, were used for this tissue and throughout the present studies.

(a) *Inherent tone* A zig-zag strip preparation was set up in Krebs-Henseleit solution at 37°C in a 10 ml bath aerated with carbogen. This preparation exhibits inherent tone due to the generation of endogenous prostaglandins (Orehek *et al.*, 1973), consequently it was left to equilibrate for 1 h before cumulative dosing with TR4979, PGE_1 or PGE_2. As only one drug could be evaluated on each preparation, paired preparations were used to decrease variability between preparations. At the end of the experiment isoprenaline was added to determine the maximal relaxant response for normalization of data.

(b) *Histamine-induced tone* Paired preparations were set-up as previously described. Indomethacin (3×10^{-6}M) was added to the bath fluid to inhibit endogenous prostaglandin synthesis and to reduce inherent tone to zero. The maximal contractant response of the preparation was determined using histamine. A concentration of histamine which produced 50% maximal response was selected and this response was shown to be maintained for over 1 h. Test drugs were evaluated for inhibition of this histamine response using a cumulative dosing procedure.

Cat trachea Specific pathogen-free cats (Category III) of either sex from the Laboratory Animals Centre were used for this and all other cat tissues in the present studies.

Tracheal rings were set up as described by Apperley *et al.* (1979), responses were measured with Harvard isotonic transducers with a load of 250 mg. An equilibration period of one hour was allowed before a stable maintained contraction was induced by a submaximal concentration of 5-hydroxytryptamine (5-HT). Test drugs were evaluated for inhibition of this contraction using a cumulative dosing procedure.

Human bronchial muscle Helical spirals of human bronchial muscle obtained from patients undergoing surgery for carcinoma of the lung were set up as described by Sweatman & Collier (1968). Responses were measured using a Harvard isotonic transducer with a load of 250–500 mg. Test drugs were evaluated by a cumulative dosing procedure and normalized to maximal isoprenaline-induced relaxation or histamine-induced contraction.

Guinea-pig lung strip The preparations were set up according to the method of Lulich *et al.* (1976) and mounted for cascade superfusion using Krebs bicarbonate solution (Vane, 1964). Responses were measured with a Harvard isotonic transducer with a load of 1 g. Histamine was used to determine the maximal contraction of the preparation and as only one drug could be tested per preparation, a vehicle control and test drug were evaluated on paired preparations.

Test drugs, or their vehicle controls in Krebs solution were continuously superfused over the

preparations and 15 min later increasing bolus doses of leukotriene D_4 (LTD_4) were added until a maximal response was achieved.

Respiratory isolated tissues containing prostaglandin 'χ' (contractant)-receptors

Indomethacin-treated guinea-pig trachea This preparation was set up as in the previous section with indomethacin (3×10^{-6}M) added to the bath fluid to inhibit endogenous prostanoid synthesis and inherent tone. Test drugs were then added to the preparation using a cumulative dosing procedure and responses normalized as a percentage of the maximal $PGF_{2\alpha}$-induced contraction.

Cat lung strip Parenchymal lung strips were prepared and set up as described by Lulich *et al.* (1976). Maximal tissue contraction was determined with 5-HT. Upon returning to baseline after washout, the relevant concentration of 5-HT was used to induce a 50% maximal sustained contraction. Test drugs were then separately added, using a cumulative dosing procedure and inhibition or potentiation of the 5-HT-induced response determined.

(2) *Non-respiratory isolated tissues containing 'χ' (contractant/stimulant)-receptors*

Pregnant/pseudopregnant uterus – human, rat and guinea-pig Pregnant human uterus (obtained from a patient undergoing hysterectomy for fibroids at the 10th week of pregnancy) and pseudopregnant rat (Wistar 150–200 g) and guinea-pig uteri (induced by stilboestrol or oestradiol 1 mg kg^{-1} s.c. 18–38 h before experiment) were set up in Krebs, De Jalon's and Van Dyke and Hastings solutions, respectively, at 30–32°C aerated with carbogen according to the method of Whalley & White (1980). Tissue movement was monitored with Harvard isotonic transducers with a load of 250–500 mg. Drugs were added sequentially to the rat uterus but cumulatively to the other two tissues. Acetylcholine and $PGF_{2\alpha}$ were used to determine the maximal contraction of the rat and guinea-pig uteri respectively.

Rat fundus, colon and chick ileum Rat fundic strip, colon and chick ileum were set up (according to the methods of Vane, 1957; Regoli & Vane, 1964; Coleman *et al.*, 1980b) in a cascade system and superfused with Krebs bicarbonate solution at a rate of 5 ml min^{-1} at 37°C aerated with carbogen. A mixture of combined antagonists (hyoscine 0.1 μg ml^{-1}, mepyramine 0.1 μg ml^{-1}, phenoxybenzamine 0.1 μg ml^{-1}, propranolol 3 μg ml^{-1} and methysergide 0.2 μg ml^{-1}) and indomethacin 3×10^{-6}M were added to the Krebs to prevent endogenous prostanoids or other common agonists being released and interacting with the tissues.

Maximal responses were obtained for PGE_2 and all the data normalized against these responses. Drugs were injected directly onto the top tissue as sequential bolus doses. The positions of the three tissues were varied between experiments.

Guinea-pig ileum The guinea-pig isolated ileum was prepared according to the method of Horton & Main (1963) and set up in a 10 ml tissue bath containing Tyrodes solution and indomethacin 3×10^{-6}M at 37°C aerated with air. Tissue movement was recorded with an isotonic transducer with a load of 1 g. Maximal tissue contraction was determined using histamine and responses normalized against it. Test drugs were evaluated initially for direct activity on the resting tension of the preparation using a cumulative dosing procedure. This was followed by evaluation of potential antagonist activity by addition of test drug to the tissue 5 min before increasing the cumulative concentrations of PGE_1 or histamine.

Drugs

The following drugs were used: acetylcholine bromide and histamine acid phosphate (BDH), indomethacin, (±)-isoprenaline sulphate, diethyl-stilboestral diproprionate and 5-hydrotryptamine creatinine sulphate (Sigma), methysergide bimaleate (Sandoz), mepyramine maleate (May & Baker), hyoscine hydrochloride (R. Daniel & Son), phenoxybenzamine hydrochloride (SKF), propranolol hydrochloride (I.C.I.), salbutamol (Allen & Hanburys), 11α,9α-epoxymethano-prostaglandin H_2 (U46619; Upjohn), PGE_1, PGE_2, $PGF_{2\alpha}$, PGD_2, PGA_1 (Ono), ICI81008 (Fluprostenol was a generous gift from Dr R.G. Bolton, I.C.I); PGI_2, $PGF_{2\beta}$ and TR4979 (Dr H. Kluender, Miles Laboratories, Elkhart), leukotriene D_4 (LTD_4) (Dr T.S. Abram, Miles Laboratories, England).

Statistical analysis

Dose-response slopes were analysed to give the dose required to produce a specified response (ED_{50}, ID_{50}) using a linear regression analysis (method of least squares). Relative potencies were also derived from complete dose-response slopes using linear regression analysis. Other statistical tests used were the paired *t* test and Fishers Exact Probability test.

Results

The wide range of respiratory and non-respiratory tissues selected for this study are purported to contain

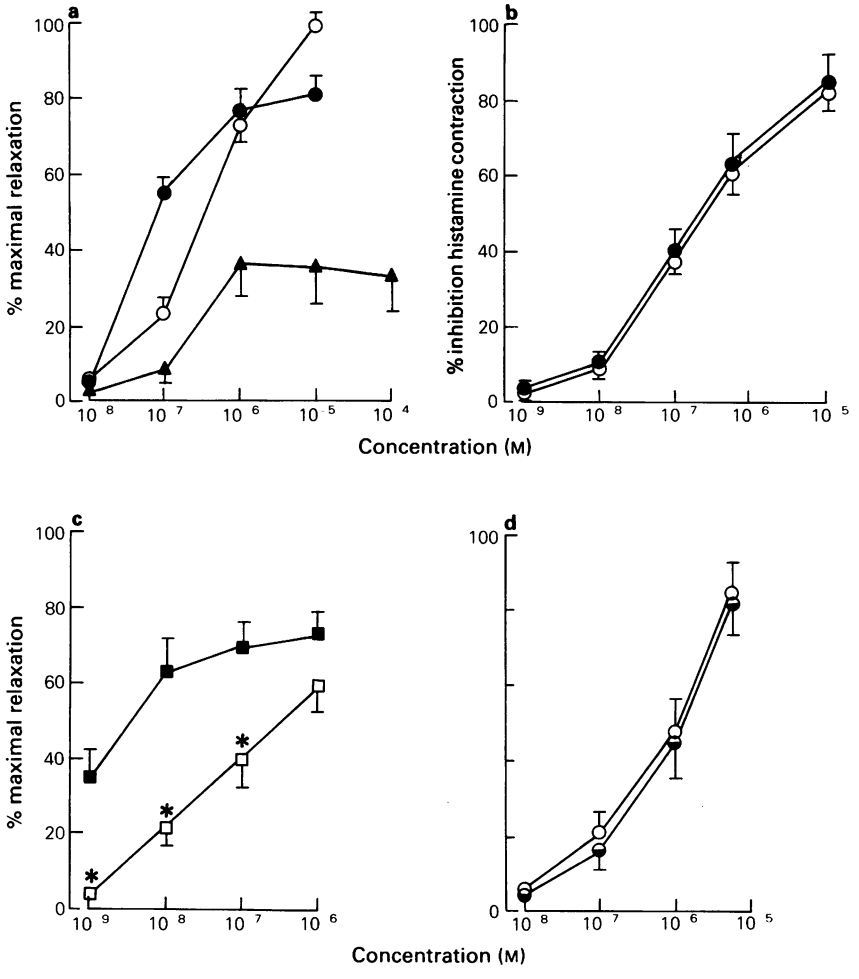


Figure 2 Activity of TR4979 on guinea-pig isolated trachea. (a) Trachea with normal inherent tone, prostaglandin E_1 (PGE_1; ●), PGE_2 (▲), TR4979 (○). Relaxant responses are expressed as % maximal isoprenaline-induced relaxation. (b) Trachea with 50% maximal tone induced by histamine in the presence of indomethacin 3×10^{-6}M, PGE_1 (●), TR4979 (○). Results are expressed as a % inhibition of the histamine-induced response. (c and d) Trachea with basal inherent tone: isoprenaline (■), TR4979 (○) alone; and isoprenaline (□) and TR4979 (○) in the presence of propranolol 1 $\mu g\,ml^{-1}$. Responses are expressed as % maximal isoprenaline-induced relaxation. Each point is the mean of 8 observations and vertical lines represent 1 s.e.mean. $^*P < 0.01$. Student's paired t test.

all the putative classes and subdivisions of prostanoid receptors currently identified. Wherever possible in the present studies a range of prostanoids were used to verify the existence of such receptors. Generally additional confirmation using selective antagonists was not possible due to lack of materials, Consequently the relevant references demonstrating the existence of such receptors are given.

Respiratory tissues

(1) *Prostaglandin 'ψ'-receptor mediated relaxant effects*

Guinea-pig trachea ('ψ', 'χ_1' and 'χ_2'-receptors – Gardiner & Collier, 1980; Copas et al., *1981a,b)*

(a) *Inherent tone* PGE_1 was a potent relaxant of the inherent tone of this tissue producing a maximal

reduction of 80% (Figure 2a). In contrast PGE_2 appeared to be a partial agonist producing a maximal relaxation of only 40%.

TR4979 had a similar profile of activity to PGE_1 i.e. an approximate 30 s refractory period, 5 min to achieve maximal response and 3–4 h washout time. The IC_{50} for TR4979 was approximately 5×10^{-7}M compared with 10^{-7}M for PGE_1. Regression line analysis of this data showed that TR4979 was 0.53 (95% confidence limits 0.32–0.88) times as potent as PGE_1.

(b) *Histamine-induced tone* The same tissue was treated with indomethacin to produce a more controlled preparation with no variable levels of inherent tone. Histamine was then used to raise tone artificially. Under these conditions cumulative dosing with PGE_1 or TR4979 produced similar dose-related relaxations of the tissue. The resultant potency by regression line analysis for TR4979 relative to PGE_1, was 0.83 (0.42–1.6) (Figure 2b).

(c) *Propranolol-treated trachea* The previous test (a) using the tissue at its normal inherent tonal level was extended and propranolol (1 μg ml^{-1}) was added to the preparation following washout of test drugs. Approximately 30 min later isoprenaline, PGE_1 and TR4979 were tested again for relaxant activity (Figure 2c and d). As expected isoprenaline-induced responses were significantly (paired t test $P < 0.01$) shifted to the right but responses to PGE_1 and TR4979 were unaffected.

Cat trachea ('ψ'-receptors – Apperley et al., *1979)* PGE_2, PGE_1 and TR4979 were all tested initially over a wide concentration range (10^{-8}–10^{-5}M) for direct activity on this tissue but no effects occurred. However, 5-HT-induced submaximal contractions were reduced by separate cumulative dosing with all three prostanoids (Figure 3). PGE_1 and PGE_2 had comparable IC_{50} values (concentration producing a 50% inhibition of the contractant response), of 2×10^{-8}M (95% confidence limits 6×10^{-9}–5.8×10^{-8}M), and 2.7×10^{-8}M,(6×10^{-9} –1.2×10^{-7}M) respectively, whereas TR4979 was weaker 4.5×10^{-7}M (9.1×10^{-8}–2.2×10^{-6}M) with a relative potency by regression line analysis to PGE_1 (= 1) of 0.04.

Human bronchial muscle ('ψ' and '$\chi_{1,3}$'-receptors, Gardiner & Collier, 1980) In some experiments indomethacin 3×10^{-6}M was added to the Krebs medium but unlike the reduction in inherent tone seen on guinea-pig trachea, it had no effect or slightly increased tone suggesting that endogenous prostanoids had little or no role in the maintenance of such tone. It also had no effect on subsequent prostanoid responses. PGE_1 and

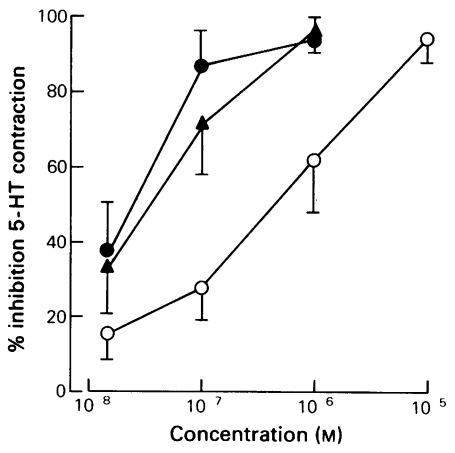


Figure 3 Effect of TR4979 on 5-hydroxytryptamine (5-HT)-induced contractions of cat isolated trachea. Prostaglandin E_1 (PGE_1; ●), PGE_2 (▲) and TR4979 (○) were added cumulatively in the presence of a concentration of 5-HT producing a submaximal contraction. Results are expressed as % inhibition 5-HT induced contraction. Each point is the mean of 4 observations and vertical lines represent 1 s.e.mean.

TR4979 produced dose-related reductions in the inherent tone of the tissue (Figure 4). Although their regression lines were significantly non-parallel, a comparison of their IC_{50} values suggested that TR4979 was 6 times less potent than PGE_1 in relaxing this tissue. In complete contrast to both PGE_1 and TR4979, dose-related contractions were induced by PGE_2 as observed in earlier studies with this tissue (Gardiner, 1975).

Guinea-pig lung strip ('ψ' and 'χ_1'-receptors – Coleman et al, *1981 and present studies)* LTD_4 was a potent contractant of guinea-pig lung strips (Figure 5). Salbutamol produced a dextral shift of the LTD_4 dose-response curve but this was not dose-related. PGE_1 was relatively inactive against these LTD_4-induced contractions, whereas TR4979 had a good inhibitory profile, producing a dose-related dextral shift with approximately 50% inhibition of the maximal LTD_4-induced response at 10^{-5}M.

(2) *Prostaglandin 'χ'-receptor mediated contractant effects*

Indomethacin-treated guinea-pig trachea ('χ_1' and 'χ_3'-receptors – Gardiner & Collier, 1980; Copas et al., *1981a, b; Jones* et al., *1982)* Indomethacin abolished the inherent tone of the tissue and in this state PGE_2

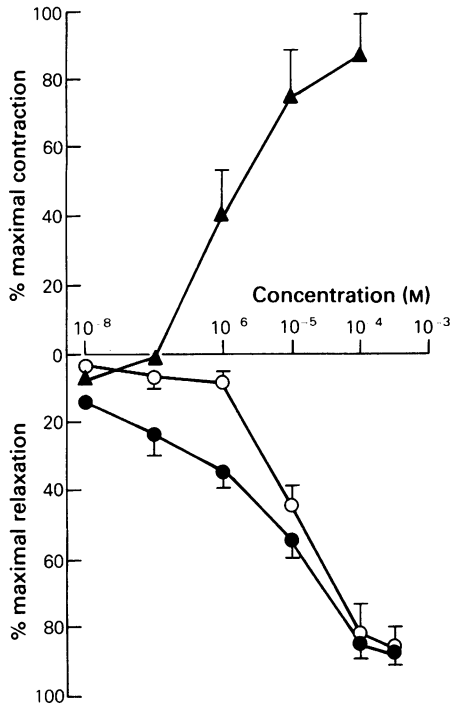


Figure 4 Activity of TR4979 on human isolated bronchial muscle. Responses to prostaglandin E_1 (PGE_1; ●) and TR4979 (○) are expressed as % maximal isoprenaline-induced relaxation whereas responses to PGE_2 (▲) are % maximal $PGF_{2\alpha}$-induced contraction. Each point is the mean of 8 observations and vertical lines represent 1 s.e.mean.

and to a lesser extent PGE_1 produced dose-related contractions of the preparation at lower concentrations than those of $PGF_{2\alpha}$ (Figure 6). Unlike $PGF_{2\alpha}$, however, both PGE_2 and PGE_1 had bell shaped dose-response curves with maximal contractions at approximately 3×10^{-8} and 10^{-7}M respectively, but such effects were at best only 60% of the maximal $PGF_{2\alpha}$-induced contractant response. In complete contrast, TR4979 had no contractant activity over a wide concentration range (10^{-9}–10^{-5}M).

Cat lung strip ('χ_{1m3} and 'ψ'-receptors – Copas et al., *1981b and present studies)* A wide range of prostanoids were tested on this tissue in the presence of 5-HT. All produced dose-related contractions with U46619 being the most potent (Figure 7). In complete contrast TR4979 (10^{-7}–10^{-5}M) was the only prostanoid which produced dose-related inhibition of the 5-HT-induced contraction.

Non-respiratory tissues

Human pregnant uterus Due to the extreme scarcity of tissue it was only possible to perform this experiment on six preparations from one sample. A wide range of prostanoids were separately added to this tissue and all contracted it to a similar degree to acetylcholine (Figure 8). TR4979 was added cumulatively to the tissue over a wide concentration range (10^{-7}–10^{-5}M) but on no occasion was a contraction observed. Instead, there was a slight reduction in the inherent tone of the preparation.

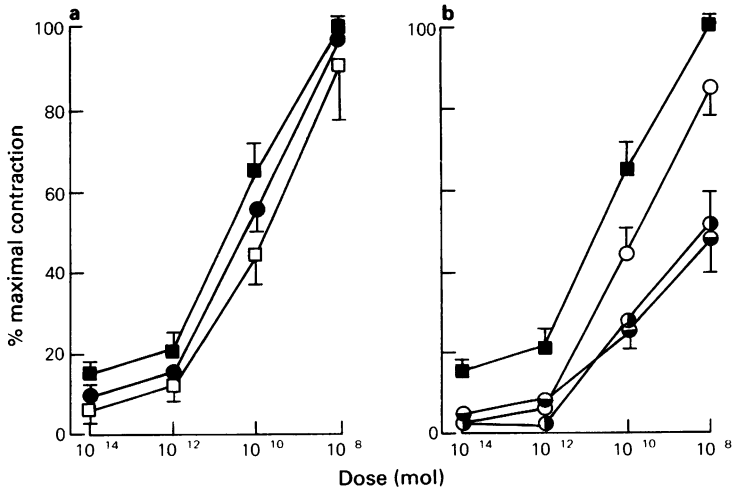


Figure 5 Inhibition of leukotriene D_4 (LTD_4)-induced contractions of guinea-pig lung strips. (a) (■) LTD_4, (●) LTD_4 + PGE_1 (10^{-6}–3×10^{-5}M), (□) LTD_4 + salbutamol 10^{-5}M. (b) (■) LTD_4, LTD_4 + TR4979 (○) 3×10^{-6}, (◑) 10^{-5} and (◒) 3×10^{-5}. Each point is the mean of 4 observations and vertical lines represent 1 s.e.mean.

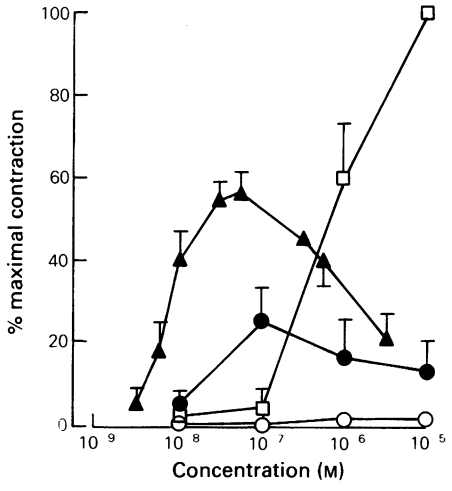


Figure 6 Effect of TR4979 on the guinea-pig isolated trachea in the presence of indomethacin 3×10^{-6}M. Prostaglandin E_2 (PGE_2; (▲), PGE_1 (●), $PGF_{2\alpha}$ (□) and TR4979 (○), were added cumulatively to the tissue. Each point is expressed as a % of the maximal $PGF_{2\alpha}$-induced contraction and is the mean of 6 observations; vertical lines represent 1 s.e.mean.

Rat and guinea-pig pseudopregnant uterus ('χ_2' and 'χ_3'-receptors – Whalley & White, 1980 and present studies) It can be seen in Table 1 that TR4979 was inactive at all doses tested on the rat uterus. In contrast the other E-type prostaglandins were potent contractants as were an additional range of prostaglandins. The rank order of contractant potency for these prostaglandins was ICI81008 > $PGF_{2\alpha}$ > PGE_2 > PGE_1 > PGI_2 > PGD_2 > PGA_1 which agrees with the findings of Whalley & White (1980) suggesting that 'χ_2'-receptors are predominant on this tissue.

The same prostanoids were evaluated on the guinea-pig uterus and all contracted it but with a different rank order of potency to the rat ($PGF_{2\alpha}$ > PGE_2 > PGD_2 > PGA_2 >> PGI_2 > ICI81008; Table 2). ICI81008 (10^{-8}–10^{-5}M) was virtually inactive on this tissue, confirming the suggestion by Whalley & White (1980) that 'χ_3'-receptors are predominant on guinea-pig uterus. TR4979 was inactive.

Rat fundus and colon ('$\chi_{1,2,3}$'-receptors – Bennett & Posner, 1971 and present studies) A wide range of prostaglandins was tested and all produced dose-related contractions (Table 3). The tissues differed slightly in that the rat colon was more sensitive to $PGF_{2\alpha}$ and ICI81008 than the fundus. This suggests that both 'χ_2'- and 'χ_3'-receptors are present on both tissues and it seems likely from the studies of Bennett

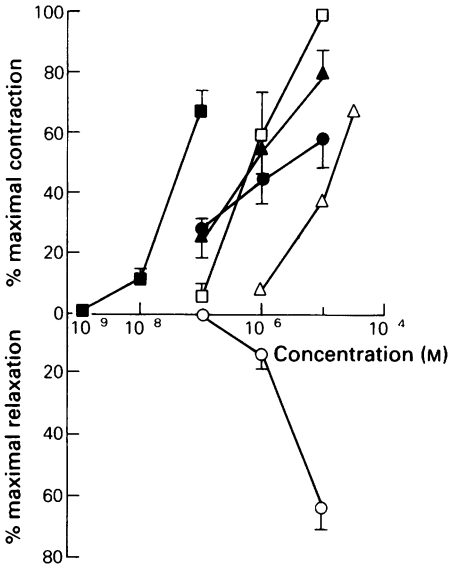


Figure 7 Relaxation/inhibition of 5-hydroxytryptamine (5-HT)-induced contraction of the cat isolated lung strip. Submaximal 5-HT responses were obtained with 5×10^{-5}M to 4×10^{-4}M in different preparations. U46619 (■), $PGF_{2\alpha}$; (□), PGE_2 (▲), PGE_1 (●) and PGI_2 (△) all induced further contractions of this tissue. Responses are expressed as % maximal 5-HT-induced contraction. TR4979 (○), relaxed/inhibited 5-HT-induced contractions. Each point is the mean of 4 observations except for PGI_2 where only 2 observations were made. Vertical lines show 1 s.e.mean.

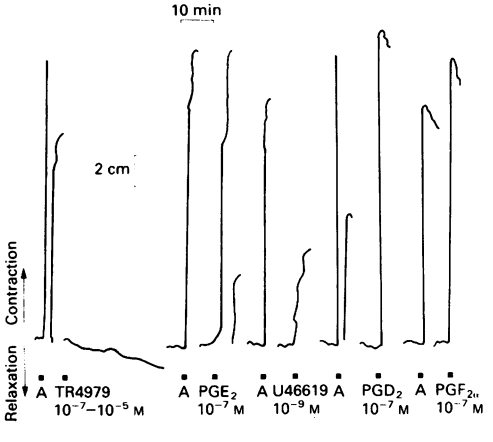


Figure 8 Activity of TR4979 on a human isolated pregnant uterus. A, represents acetylcholine. Each prostaglandin was tested on a separate preparation taken from the same tissue sample. TR4979 was added cumulatively and produced a reduction of inherent tone.

Table 1 Activities of TR4979 and some prostaglandins on pseudopregnant rat uterus

Conc.		*% maximal $PGF_{2\alpha}$ contraction*			
(M)	n	*$PGF_{2\alpha}$*	*PGE_2*	*PGE_1*	*TR4979*
10^{-8}	8	5.2 ± 2.4	1.0	0	0
10^{-7}	8	32.4 ± 8.8	5.5 ± 1.4	2.3 ± 1.1	0
5×10^{-7}	8	63.3 ± 6.7	24.3 ± 3.5	29.4 ± 8.9	0
10^{-6}	8	72.8 ± 3.7	46.4 ± 5.8	38.5 ± 6.8	0
5×10^{-6}	8	93.1 ± 3.5	86.3 ± 5.7	64.9 ± 10.0	0

All responses were normalised as a percentage of the maximal response to $PGF_{2\alpha}$ (10^{-5}M) and the data show mean ± s.e.mean. All compounds were evaluated on eight separate preparations (*n* value) but the order of testing was varied.

Table 2 Activities of TR4979 and some prostaglandins on pseudopregnant guinea-pig uterus

Conc.		*% maximal $PGF_{2\alpha}$ response*			
(M)	n	*$PGF_{2\alpha}$*	*PGE_2*	*PGE_1*	*TR4979*
10^{-8}	8	7.3 ± 1.3	35.3 ± 11.9	7.9 ± 1.4	0
2.5×10^{-8}	8	33.3 ± 4.5	38.3 ± 12.2	n.t.	0
5.0×10^{-8}	8	61.8 ± 5.3	48.0 ± 17.8	11.3 ± 3.7	0
10^{-7}	8	80.6 ± 3.6	57.3 ± 17.2	n.t.	0
5.0×10^{-7}	8	99.8 ± 0.2	78.2 ± 12.9	n.t.	0

Normalisation, procedure and *n* values as for Table 1. ICI81008 was relatively inactive on this tissue suggesting that little if any 'χ_2'-receptors are present. n.t. – not tested.

Table 3 Activities of a range of prostanoids on rat gastrointestinal tissues

		Relative potency (95% confidence limits)	
	n	*Rat fundic strip*	*Rat colon*
PGE_2	8	1.0	1.0
$PGF_{2\alpha}$	8	0.31 (0.15–0.62)	0.53 (0.005– 3.7)
PGE_1	8	0.09 (0.04–0.21)	0.025 (0.002–17.0)
ICI81008	8	0.08 (0.01–0.27)	0.34 (0.16 – 0.7)
$PGF_{2\beta}$	8	0.03 (0.01–0.07)	0.01 (0.002– 0.05)
TR4979	8	*<0.0001	★<0.0001

Relative potency was determined by regression line analysis and comparison with PGE_2 (= 1), *n*, represents the number of preparations. The order of test drug administration was varied. ★TR4979 (2×10^{-9}–2×10^{-4}mol) was inactive.

Table 4 Activities of TR4979 and a range of prostanoids on the chick ileum

	ED_{50} (95% confidence limits) (μmol)	★ *Relative potency*
PGE_2	0.015 (0.008– 0.12)	1
PGE_1	0.076 (0.01 – 0.19)	0.2
$PGF_{2\alpha}$	0.40 (0.15 – 0.85)	0.04
PGI_2	0.63 (0.41 – 1.1)	0.02
PGA_1	8.5 (1.3 –15.5)	0.002
TR4979	>100.0	<0.001

★Relative potency was determined from the ED_{50} (dose producing a 50% maximal PGE_2 contractant response) values. All results were from eight separate preparations with varied ordering of test drug administration.

et al. (1978), using U46619, that 'χ_1'-receptors are also present although we were unable to test this possibility due to scarcity of material. TR4979 (2×10^{-9}–2×10^{-4}mol) was inactive on both tissues.

Chick ileum ('ψ'-receptors – Coleman et al., *1980b)* The receptor purported to be present on this tissue is mainly sensitive to the E-prostaglandins but is insensitive to the antagonist SC19220, characteristics which suggest that it is similar to the relaxant receptor on cat trachea. The rank order of potency obtained in this study corresponded to that on cat trachea, although $PGF_{2\alpha}$ was more active than had been expected (Table 4).

However, in contradiction to Coleman's hypothesis, TR4979 was inactive over a wide dose range (2×10^{-9}–2×10^{-4}mol) on this tissue.

Guinea-pig ileum TR4979 produced small (<20% maximal histamine contraction) contractions of approximately half the preparations at 10^{-6}M but was inactive at 10^{-5}M suggesting that it had a bell shaped dose-response curve on some tissues. In the second part of the experiment, TR4979, 3×10^{-6}M and 10^{-5}M non-specifically inhibited PGE_1- and histamine-induced responses in a non-competitive manner, suppressing their normal maximal responses by almost 80–90% at the highest concentration used (Figure 9).

Discussion

TR4979 is a potent relaxant of airway smooth muscle, from man, guinea-pig and cat, with inherent or pharmacologically-induced tone. The lack of effect of propranolol against such activity and the similar characteristics of this relaxant activity to that of PGE_1 suggests that TR4979 is acting via a distinct prostanoid receptor(s) which has previously been given the putative classification of the 'ψ'-receptor (Gardiner & Collier, 1980). Such a profile of activity is perhaps not surprising considering the compounds structural similarity to PGE_1. What was surprising, however, was its high selectivity for the 'ψ'-receptor relative to PGE_1. Such a conclusion was drawn from the evaluation of TR4979 on the following range of test tissues, purported to contain the spectrum of the prostanoid class of '$\chi_{1,2,3}$'-receptors responsible for contractant/stimulant activity.

The 'χ_1'-receptor is principally stimulated by TXA_2, as demonstrated with a thromboxane mimetic U46619. In the present study such a receptor was predominant in guinea-pig lung and was present as part of a heterogeneous prostanoid receptor population with 'χ_3'-contractant receptors and 'ψ'-relaxant receptors on guinea-pig trachea and cat lung strip (Coleman *et al.*, 1981; Copas *et al.*, 1981a, b; Jones *et al.*, 1982). Although the naturally occurring prostaglandins were either inactive (PGE_1) or contracted the guinea-pig lung strip to varying degrees, TR4979 was a consistent relaxant which was more effective than the selective β_2-adrenoceptor agonist, salbutamol. On guinea-pig trachea, either at inherent or induced tonal states, TR4979 again acted as a consistent potent relaxant. Experimental conditions for the latter tissue can be manipulated using indomethacin to abolish inherent tone, enabling only the contractant activity of prostaglandins to be expressed via the 'χ_3'-

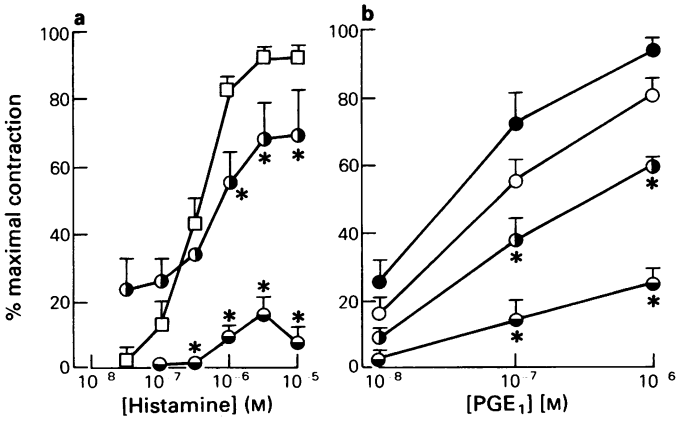


Figure 9 Inhibition of (a) histamine or (b) prostaglandin E_1 (PGE_1)-induced contractions of guinea-pig isolated ileum by TR4979. (a) Histamine was added to the tissue alone (□) and in the presence of TR4979 3×10^{-6}(◑), and 10^{-5} (◒)M. (b) PGE_1 was also added to the tissue alone (●) and in the presence of TR4979 10^{-6}(○), 3×10^{-6} (○) and 10^{-5} (◒)M. Each point is the mean of 4–6 observations and vertical lines represent 1 s.e.mean. Responses are expressed as a % of the maximal histamine-induced contraction. *$P < 0.05$, Student's paired t test.

receptor (Gardiner & Collier, 1980). Under these conditions PGE_2, and to a lesser extent PGE_1, had significant contractant activity, although their dose-response curves were bell shaped, presumably due to their relaxant activities being revealed at higher tonal levels. TR4979, however, was inactive under these conditions. Similarly, on cat lung strip TR4979 was a consistent relaxant, an effect not observed with any of the other prostaglandins.

The 'χ_2'-receptor is mainly stimulated by $PGF_{2\alpha}$ or its analogue IC81008 and in the present study was predominant on rat uterus and represented one of the heterogeneous receptor populations on the rat colon and human bronchial muscle (Gardiner & Collier, 1980; Whalley & White, 1980). $PGF_{2\alpha}$ and ICI81008 were potent contractants of these tissues, whereas TR4979 was either inactive, as seen on the rat tissues, or acted in the opposite way relaxing the tissue as seen with human bronchial muscle. Although the latter effect was also observed for PGE_1, no such similarity was seen on the rat tissues where PGE_1 was a potent contractant.

The 'χ_3'-receptor is characterized by the agonist PGE_2 and to a lesser degree PGE_1 and is competitively antagonized by SC19220 (Bennett *et al.*, 1978). It is predominant on the guinea-pig ileum and uterus and constitutes part of a heterogeneous population on indomethacin-treated guinea-pig trachea, human bronchial muscle, rat fundus and cat lung strip (Bennett *et al.*, 1978; Whalley & White, 1980; Copas *et al.*, 1981a,b). Although PGE_2 and to a lesser degree PGE_1 were potent contractants of all these tissues, TR4979 had little or no activity over a ten thousand fold concentration range.

Taken together the previous results suggest that TR4979, unlike its parent compound PGE_1, is extremely selective for prostanoid 'ψ'-receptors with no demonstrable contractant '$\chi_{1,2,3}$'-receptor mediated activity on a range of respiratory or non-respiratory tissues. Such selectivity is at present unique for any prostaglandin agonist but demonstrates that it can be achieved with prostaglandins in a comparable way to selective adrenoceptor or histamine agonists.

Further studies with this compound on the guinea-pig ileum suggested that its relaxant activity is not restricted to airway smooth muscle and that by inference populations of prostanoid 'ψ'-receptors also exist on non-airway smooth muscle. Although it had little or no activity alone on the guinea-pig ileum, TR4979 non-specifically antagonized contractions induced by PGE_1 or histamine. Such an effect was dose-related and unaffected by propranolol 1 $\mu g\,ml^{-1}$ (personal communication J. Browne). It seems likely from such a finding that this tissue also contains 'ψ' (relaxant)-receptors but that PGE_1 and PGE_2 selectively stimulate the predominant 'χ_3'-receptor(s) resulting in an overall contractant response. The existence of 'ψ'-receptors on this tissue has already been proposed by Bennett *et al.* (1968) to explain the relaxant effects of PGE_2 on gastrointestinal circular smooth muscle. Consequently it seems possible that TR4979 is acting solely on 'ψ'-receptors on the circular smooth muscle of guinea-pig ileum in preference to the 'χ'-receptors on the longitudinal muscle. Unlike previous studies, where circular muscle and longitudinal muscles had to be separated to show such distinct and opposing actions/receptors of prostaglandins, differentiation can be achieved on the whole tissue preparation using TR4979.

The profile of action of TR4979 on the chick ileum seems to question a part of the receptor classification proposed by Coleman *et al.* (1984). They suggested that the prostanoid receptor responsible for relaxation of the cat trachea and contraction of the chick ileum was the same and that both were insensitive to the prostaglandin antagonist SC19220. In contradiction of this, however, TR4979 was a potent relaxant of the cat trachea but was inactive on the chick ileum. Using Coleman's classification TR4979 would be expected to act as a potent contractant of chick ileum. It seems more appropriate using the present receptor classification to include PGE actions on chick ileum as distinct from those on cat trachea. Perhaps they are mediated by 'χ_3'-receptors. The ineffectiveness of SC19220 against such a response (Coleman *et al.*, 1980b), however, suggests that a further subdivision in the classification of this receptor class may exist.

The high degree of selectivity of TR4979 for one particular action of prostanoids, namely relaxation/inhibition, strengthens the earlier suggestion that receptor classification be made according to the prostanoids action and that overall two distinct receptor classes exist. Although only a few prostanoid antagonists have as yet been identified, they all strengthen the two class receptor hypothesis as they show much lower selectivity for prostanoids with contractant/stimulant actions than for the opposing relaxant/inhibitory actions i.e. B.M. 13.177 antagonizes U46619 and $PGF_{2\alpha}$-induced contractions of rat and rabbit aorta, trimetequimol antagonizes both U46619 and PGE_2-induced contractions of rat aorta, SKF 88046 antagonizes TXA_2, PGD_2 and $PGF_{2\alpha}$-induced contractions of guinea-pig trachea and SC19220 antagonizes PGE_2 and $PGF_{2\alpha}$-induced contraction of guinea-pig trachea but all are inactive against prostanoid relaxant activity (Weichman *et al.*, 1984; Patscheker *et al.*, 1984; Mukopadhyay *et al.*, 1985). It seems appropriate that only one distinct receptor class represents all of the similar activities (contraction/stimulation) of prostanoids and that subclasses be used to expiain the smaller differences within this class.

At present the unique prostanoid receptor selectivity of TR4979 provides a valuable new tool for

gaining information of the potential therapeutic or pathophysiological role of the 'ψ'-receptor. The lung and gut have already been mentioned in this respect. However, prostanoids have activities on numerous other tissues but their ability to stimulate more than one prostanoid receptor has as yet made any useful interpretation of such results virtually impossible. It is tempting to speculate that 'ψ'-receptors may have additional beneficial effects other than bronchodilatation e.g. in the cardiovascular system, pain or inflammation. It remains to be seen, however, whether such systems possess the 'ψ'-receptor and/or whether it exists as further subdivisions. Our preliminary studies of additional 'ψ'-receptor systems i.e. vasodilatation *in vivo* and inhibition of human platelet aggregation *in vitro* using PGE_1, PGE_2, PGI_2 and TR4979 suggest, that subdivisions of this receptor class do occur (Copas *et al.*, 1981c).

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